DESCRIPTION OF A NEW SPECIES IN GENUS *THRAULODES* (EPHEMEROPTERA: LEPTOPHLEBIIDAE) AND AN ANALYSIS OF COMPARITIVE LEG MORPHOLOGY AMONG MULTIPLE SPECIES

An Undergraduate Research Scholars Thesis

by

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Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. David E. Baumgardner

May 2017

Major: Zoology

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ABSTRACT

Description of a New Species in Genus *Thraulodes* (Ephemeroptera: Leptophlebiidae) and an Analysis of Comparative Leg Morphology among Multiple Species

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The genus *Thraulodes* (Ephemeroptera: Leptophlebiidae) is widely distributed across the Americas, and can be found in lotic systems throughout North, Central, and South America (Chacón et. al, 1999). With 45 described species, it is one of the most speciose genera in the family Leptophlebiidae. Despite the widespread abundance, this genus has been poorly documented with many species described as adults but lacking description of their larval counterpart. A morphological comparison between larval legs of five species will be presented and evaluated for taxonomic significance, along with the description of a new species from Guatemala.

ACKNOWLEDGEMENTS

I would like to thank Dr. David Baumgardner for providing specimens from his lab used throughout the study, as well as dragging me all over Central America. His guidance and mentorship will continue to affect my career decisions long after I leave Texas A&M.

CHAPTER I

INTRODUCTION

Mayflies (Insecta: Ephemeroptera), along with stoneflies (Plecoptera) and caddisflies (Trichoptera), are highly sensitive to pollutants in the water and serve as important bioindicators in determining water quality of an aquatic ecosystem (Menetrey et. al, 2008). Understanding the taxonomy of mayflies and other invertebrates assists in evaluating the health of an aquatic ecosystem.

Despite being a significant component of benthic fauna, *Thraulodes* remains poorly studied. The genus was established by Ulmer in 1920 based on imagos of *Thraulus laetus* from Colombia, collected by Eaton (1884). Since then, the species count has reached near 50, however almost half of these species are not associated with a nymph (Mariono et. al, 2011). A complete species key for the adults was proposed by Traver and Edmunds in 1967, which placed heavy emphasis on morphology of imago male genitalia. In 1978, Allen and Brusca published a species key for the nymphs in North and Central America. Since then there have been various contributions and the genus has grown beyond the scope of the key.

The purpose of this study is to describe a new species of *Thraulodes* and provide a morphological taxonomic evaluation of the larval legs of *Thraulodes lunatus, T. zonalis, T. speciosus, T. gonzalesi,* and *T. brunneus.* The reason for this comparison is to investigate for differences in the larvae beyond the color patterns used by Allen and Brusca (Allen and Brusca, 1978) to evaluate species of *Thraulodes* in the larval stages. While patterns on the abdominal terga are often striking, in several species, the patterns are very similar and when combined with slight individual variation can be very difficult to use for identification. Additionally, over time

specimens preserved in ethanol lose their coloration making them unidentifiable. For some genera and species, setae and spine patterns in mouthparts have proven important in identifications (Peters, 1971). While mouthparts have been thoroughly observed, descriptions of legs are reserved to general color patterns.

CHAPTER II

METHODS

Part 1: Description of *Thraulodes cahaboneri*

Thraulodes cahaboneri was collected in the Cahabón River in Alta Verapaz, Guatemala (15.53° N, 89.94°W). Collections were made by examining benthic matter and removing Ephemeroptera larvae using forceps. Specimens were preserved in 100% ethanol and brought back to the lab for study under a dissecting microscope. Similar techniques were used for collection in Mexico. Mexican specimens were collected from the Rio Calnali in the town of Calnali, Hidalgo, Mexico.

Legs were removed and mounted using Euparol as the mounting agent. The fourth gill from the right side was removed and placed on a wet mount slide using 75% ethanol as a mounting agent. The gill length/width ratio was measured using a Nikon SMZ-1 dissecting scope with attached straight line reticule. Each gill was measured along its longest axis and widest point using an ocular micrometer. Specimens were returned to individual labeled vials containing 75% ethanol for storage.

Part 2: Descriptions of Larval Legs

The larval legs of *Thraulodes lunatus, T. zonalis, T. speciosus, T. gonzalesi,* and *T. brunneus* were on mounted slides using Euparol mounting media. Pattern, quantity, and morphology of spines and setae were observed along the femora, tibia, and tarsus of the foreleg, midleg, and hindleg for each of the aforementioned species.

CHAPTER III

RESULTS

Part 1: Description of *Thraulodes cahaboneri*

The head is brown, matching the general coloration of the specimen. Antennae are pale, and slightly longer than the head capsule. The area between the eyes is dark (Figure 1).

Figure 1. Dorsal view of the head of *Thraulodes cahaboneri*

The labium is pale. Glossae and Paraglossae with numerous short stout setae, particularly in the posterior region of the Glossae. Palps have three segments. The first segment has ~20 setae posteriorly and ~10 anteriorly. The second segment has none or very few setae. The third segment has ~10 setae (Figure 2a). The labrum is pale. Two rows of setae. One runs along the posterior edge. The other is on the dorsal face, extending posteriorly at the anterolateral emargination (Figure 2b). The maxillae have an apical tuft of setae extending past the galealacinia. There is a row of simple setae medially on the lacinia. Maxillary palps are three segmented, and extend past the galealacinia. The first segment is robust with ~10 fine setae on the lateral margin. The second segment has ~15 fine setae. The third segment is covered in fine

setae, with greater concentration at the apex (Figure 2c). Mandibles as pictured below (Figure 2d, 2e).

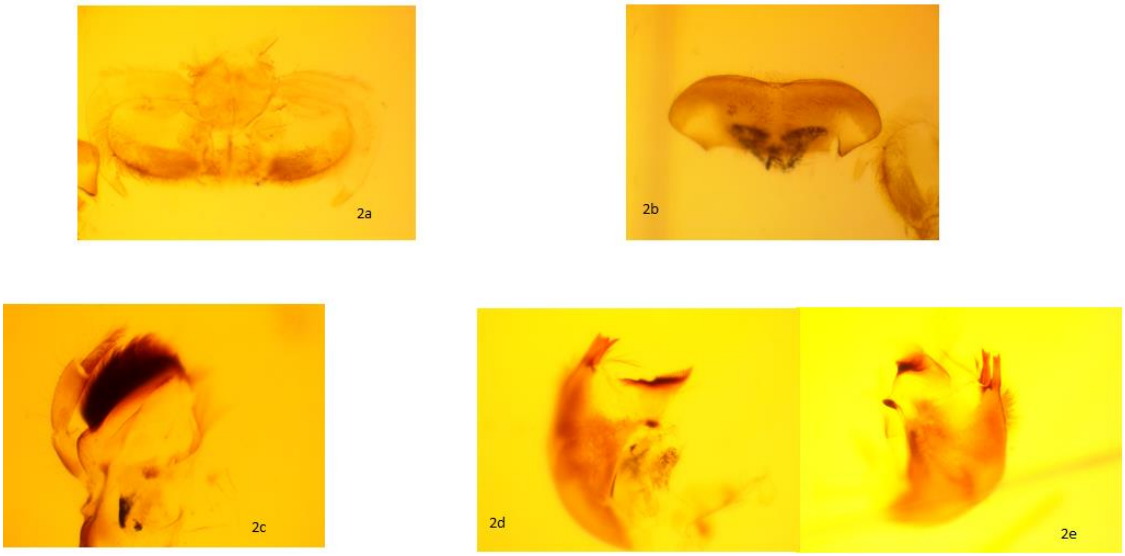

Figure 2. Mouthparts of *Thraulodes cahaboneri*. (2a) Labium. (2b) Labrum. (2c) Maxillae. (2d) Right mandible. (2e) Left mandible.

The Mesonotum is brown with extensive markings. The legs are brown with white maculae along dorsal face of femora. Usually 2-3 markings, giving the legs a banded appearance (Figures 3a,3b).

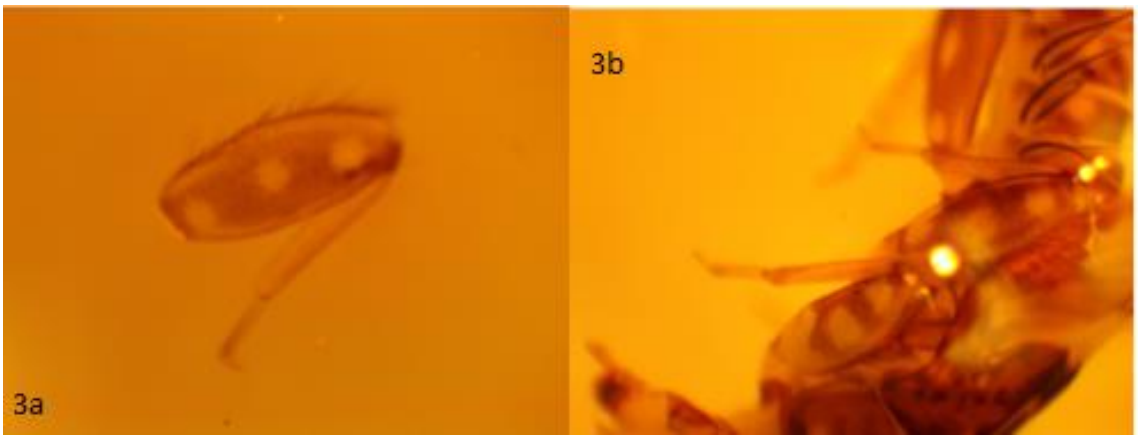

Figure 3. Coloration of legs. (3a) Maculation on an individual leg. (3b) Legs attached giving a banded appearance.

The gills have a gradual taper, indicative of the gonzalesi group (Allen and Brusca, 1978). They lack lateral trachea. Gills moderately narrow,7.24:1 ratio (Table 1) (Figure 4).

Figure 4. Gill 4 of *Thraulodes cahaboneri*

Table 1. Length/Width Ratios of Gills

	Length	Width	Ratio
JB.3.x.2016.01	4.8	.7	6.86/1
JB.3.x.2016.01	4.9	.7	7.0/1
JB.18.x.2016.03	3.9	.6	6.5/1
JB.18.x.2016.03	4.5	.5	9.0/1
JB.18.x.2016.02	4	.5	8.0/1
JB.18.x.2016.08	3.1	.5	6.2/1
JB.18.x.2016.08	3.1	.7	5.23/1
JB.18.x.2016.01	3.9	.6	6.5/1
JB.18.x.2016.01	4.0	.7	5.71/1
JB.18.x.2016.07	3.3	.5	6.6/1
JB.18.x.2016.07	3.4	.5	6.8/.1
JB.18.x.2016.06	2.7	.4	6.75/1
JB.18.x.2016.06	2.8	.3	9.33/1
JB.18.x.2016.05	3.8	.5	7.6/1
JB.18.x.2016.05	4	.5	8.0/1
JB.18.x.2016.09	3.4	.4	8.5/1
JB.18.x.2016.09	3.4	.4	8.5/1
Avg.	3.7058824	.529412	7.24/1

The Abdominal terga is brown with posterolateral white maculae. Anterolateral dark maculae may or may not be present. There is a large amorphous white spot on terga 4-5 (Figure 5). Caudal filaments are pale with dark annulations.

Figures 5. Abdominal tergum of *Thraulodes cahaboneri.* Posterolateral white maculae present, anterolateral black maculae may be present as well.

Holotype: Larva, Guatemala, Alta Verapaz Department 15.538669° N, 89.944811° W 380 M, Barnett and Baumgarnder 2016 deposited at TAMU

Paratypes: 98 larvae have been collected, same data as holotype

Etymology: We name this species after the type locality in the Cahabón River in Alta Verapaz, Guatemala.

Discussion: This species can be easily identified by the strong banding pattern in the legs, as well as the unique terga patterns. The posterolateral white maculation along with the medial white macula and pale posterior terga are unique to this species.

Part 2: Description of Larval Legs

Thraulodes lunatus

Hindleg

Femur: Setae begin halfway up the posterior edge of the femur. ~60 setae along the posterior edge. Setae along the anterior edge are much sparser (<20) and may be absent. Spines are particularly prominent. A row of ~50 spines runs along the posterior edge. Spines are observed in rows and in random assortment throughout the dorsal face. A row of short spines runs the length of the anterior edge.

Tibia: Dense setae run along the length of the posterior edge of the tibia. There are few setae along the anterior edge and may be completely absent. ~20 small spines observed in two rows along the anterior edge on both the dorsal and ventral face. Spines along the posterior edge are variable, and may be present or absent.

Tarsus and Tarsal Claw: ~20 setae observed along the posterior edge of the tarsus. There are none or few along the anterior edge. Roughly 5 spines along the anterior edge and none along the posterior. There are 6 denticles in the tarsal claw.

Midleg

Femur: Long dense setae run the length of the posterior edge, Roughly 30 long spines are present along the posterior edge. Spines are present throughout the dorsal face of the femur. Spines along the anterior edge are shorter and number in the twenties.

Tibia: Dense setae run along the length of the posterior edge of the tibia. Setae are present along the anterior but are neither as long nor as dense. Roughly 10 spines were observed along the anterior edge.

Tarsus and Tarsal Claw: 10 setae line the posterior edge of the tarsus and may be present along the anterior edge. Spines are not present. 10-11 denticles observed in the tarsal claw.

Foreleg

Femur: Setae run the length of the posterior edge with increasing density toward the tibia. Setae are completely lacking along the anterior edge. Spines line the posterior edge and are present throughout the dorsal face.

Tibia: The posterior of the tibia ~80 setae or fewer and no spines. The anterior edge has a prolific row of ~60 spines but lacks setae.

Tarsus and Tarsal Claw: The tarsus has ~60 setae along the posterior edge. These may be absent. There are 10 denticles in the tarsal claw.

Thraulodes zonalis

Hindleg

Femur: Setae begin 1/3 of the way up the posterior edge and are moderately dense. A row of short spines extends the length of the posterior, which are interspersed with longer spines toward the tibia. Spines occur throughout the dorsal face with greater concentration toward the posterior edge. The concentration of spines along the dorsal face may vary between specimens.

Tibia: Setae are present along the posterior and anterior edge with greater concentration and length on the posterior edge. In other specimens, the anterior setae were not present. Spines of varying length run the length of the posterior edge. Just anterior to this row, there is a row of

very short spines. The anterior edge has 10 spines, although this is not consistent through the observed specimens.

Tarsus and Tarsal Claw: Long setae run in high density along the tarsal claw. A conspicuous spine is located at the "ankle" between the tarsus and the claw. The claw has between 6-10 denticles.

Midleg

Femur: Long dense spines run the length of the posterior edge. These spines may vary in length. Spines are prominent on the dorsal face, increasing in density toward the posterior edge. Anterior spines are much shorter.

Tibia: Long setae run the length of the posterior edge. Roughly 10 spines along the anterior edge.

Tarsus and Tarsal Claw: Long Setae continue along the posterior edge of the tarsus. The claw has 6-10 denticles.

Foreleg

Femur: Long setae run the length of the posterior edge. The posterior edge has long spines with concentration varying between specimens. Spines along the anterior edge may be present or lacking. Spines are found on the dorsal face, but lack any sort of discernible pattern.

Tibia: Setae run the length of the posterior edge, but are not found along the anterior edge. Spines are found along the anterior edge but are lacking in the posterior edge.

Tarsus and Tarsal Claw: There are ten or fewer setae along the posterior edge of the tarsus. Spines are absent. The claw has between 6-10 denticles.

Thraulodes speciosus

Hindleg

Femur: Setae begin 1/3 of the way up the posterior edge. These are relatively sparse, numbering around 30. Setae along the anterior edge are very sparse. The posterior margin has dorsal and ventral rows of 40-50 long spines each. Spines are found in high density on the dorsal face, especially in the margins near the edge. A row of short spines lines the anterior edge.

Tibia: A row of setae run the length of the posterior edge of the tibia, with sparse setae along the anterior edge. There are dorsal and ventral rows of around 30 spines along the anterior edge. Spines along the posterior edge are variable, and may be present or absent.

Tarsus and Tarsal Claw: Less than 10 setae are present on the anterior and posterior edges of the tarsus. No spines are present. 5-7 large denticles are present in the claw.

Midleg

Femur: A dense row of setae runs the length of the posterior edge. Setae are absent along the anterior edge. A row of midlength and long spines begin halfway up the femur. Spines are found throughout the dorsal face with greater density toward the thorax. Short spines line the anterior edge.

Tibia: The posterior edge of the tibia lacks spines. A row of around 15 short spines lines the anterior edge. A row of long dense setae runs along the posterior edge. Setae are found along the anterior edge in clumps.

Tarsus and Tarsal Claw: Less than 20 setae are found along the posterior and anterior edge of the tarsus. 7-12 small denticles are present in the claw.

Foreleg

Femur: A row of long dense setae run along the posterior edge. The anterior edge lacks setae. A row of midlength and long spines lines the posterior edge with longer spines toward the

tibia. A row of short spines is present along the anterior edge. Spines cover the dorsal face, appearing in a linear fashion in the posterior margin.

Tibia: A row of mid length setae runs along the posterior edge. The anterior edge lacks setae but has dense rows of spines along the dorsal and ventral side.

Tarsus and Tarsal Claw: Less than 10 spines are along the posterior and anterior edge of the tarsus. 6 large denticles are present in the tarsal claw.

Thraulodes gonzalesi

Hindleg

Femur: A row of roughly 100 long setae begins roughly 1/3 of the way up the posterior edge. Setae may be absent along the anterior edge. Long and midlength spines form along the posterior edge, and may be shadowed by an additional row immediately anterior to the first. Short spines cover the dorsal face and line the anterior edge.

Tibia: Long dense setae run the length of the posterior edge. Setae are present along the anterior edge but are shorter and more sparse. A row of 25-40 spines lines the dorsal and ventral face of the posterior edge. Short spines are found along the anterior edge.

Tarsus and Tarsal Claw: Long setae appear in the posterior edge of the tarsus in similar density to the tibia. Spines may be present along the posterior and anterior edges. The tarsal claw has 6-10 denticles.

Midleg

Femur: Long dense setae run the length of the posterior edge of the femur. Sparse setae may be present along the anterior edge. Long and midlength spines line the posterior edge. Short spines cover the dorsal face and anterior edge with no discernible pattern.

Tibia: Setae are long and dense along the posterior edge, and are much sparser along the anterior edge (5-35). The posterior edge lacks spines. The anterior edge has short spines.

Tarsus and Tarsal Claw: There are no spines present on the tarsus. Setae are present exclusively on the anterior edge. There are between 7-10 denticles on the tarsal claw.

Foreleg

Femur: Long dense setae run the length of the posterior edge but are lacking on the anterior edge. Long and midlength spines are found along the posterior edge and immediately below. The anterior edge is void of spines. Short spines cover the dorsal face.

Tibia: Setae are sparse along the posterior edge of the tibia and lacking on the anterior edge. Rows of 15-30 curved spines line the anterior edge.

Tarsus and Tarsal Claw: Sparse setae along the posterior edge of the tarsus, no spines. 7-12 denticles on the tarsal claw.

Thraulodes brunneus

Hindleg

Femur: Setae are relatively sparse along the posterior edge of the femur, numbering in the 50s. A row of midlength spines runs the length of the posterior edge, with long spines occurring sporadically toward the tibia. Short spines cover the dorsal face, with a greater concentration in the posterior margin. There are short spines and sparse setae along the length of the anterior edge.

Tibia: Dense long setae run along the posterior edge. Along the posterior edge there are roughly 30 midlength spines and a row of around 40 short spines. Setae are short and sparse along the anterior edge.

Tarsus and Tarsal Claw: The tarsus may or may not have few spines and setae along both edges. There are between 5-8 denticles in the tarsal claw.

Midleg

Femur: A row of ~100 long setae run the length of the posterior edge. A dense row of midlength spines occurs along the posterior edge, with longer spines toward the tibia. Short spines occur through the dorsal face, with increased concentration near the posterior edge. Short spines line the anterior edge.

Tibia: The posterior edge of the tibia has long setae, but lacks spines. Along the anterior edge there is a row of between 10-50 short and midlength spines.

Tarsus and Tarsal Claw: ~10 setae along the posterior edge of the tarsus. Between 5-8 denticles in the tarsal claw.

Foreleg

Femur: Long setae run along the posterior edge of the femur. Midlength and long spines are along the posterior edge with longer spines toward the tibia. Short spines cover the dorsal face and lline the anterior edge.

Tibia: Anterior edge has sparse setae, and may or may not have a row of short spines. The posterior edge has ~40 spines along the dorsal and ventral side. Setae are present along the posterior edge.

Tarsus and Tarsal Claw: Spare setae may or may not be present along the posterior and anterior edge of the tarsus. There are between 4-6 denticles in the tarsal claw.

CHAPTER IV

CONCLUSION

Part 1: Description of *Thraulodes cahaboneri*

This species is easily distinguished from the nymphs of all other species. The clear distinctions in abdominal terga along with the strong banding pattern on the legs suggest this nymph has not been previously described. The high degree of similarity observed in roughly 100 specimens strengthens the case for an independent species not previously described.

The banding pattern on the legs was not exhibited by any of the five species examined in Part 2, and is highly unusual. It was also observed from a separate population collected in Mexico (Figure 6), however due to morphological differences in the gills and terga the two populations cannot be considered conspecific (Figure 7).

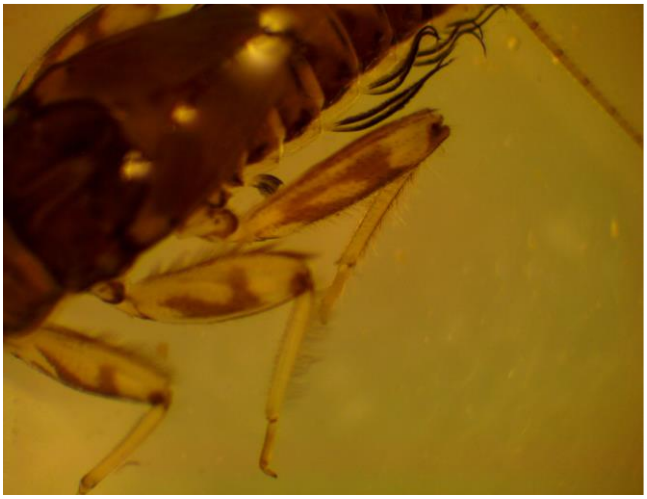

Figure 6. Banded legs from *Thraulodes sp.* Collected in Mexico. Resembles the pattern observed in *Thraulodes cahaboneri.*

Figure 7. Abdominal terga of *Thraulodes sp.* collected in Mexico. Maculation differs from *Thraulodes cahaboneri.* Additionally, gills have minor lateral tracheation further supporting the two populations as different species.

Part 2: Descriptions of Larval Legs

Both spines and setae are highly variable within the individual. As a general pattern, spines were prominent along the anterior edge and setae occurred in greater amounts on the posterior margin. Due to the high amount of variation within conspecific individuals, apomorphic traits were not identified, supporting the null hypothesis that patterns of spines and setae on the legs are not taxonomically significant.

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